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Dear Sir or Madam:

As required by 40 CFR 716, as amended, we with submit a copy of the following recently completed health and safety study.

> Determination of the effect of TDI, TDA, MDI & MDA on the emergence and growth of the plant species Avena sativa and Latuca sativa according to OECD Guideline no. 208. Project E-CE-95.

Chemical Name	CAS Number
Toluenediisocyanate	26471-62-5
Toluenediamine	25376-45-8
Polymeric diphenyl methane diisocyanate	9016-87-9
(contains 4,4'-diphenyl methane diisocyanate)	101-68-8
4,4'-diaminodiphenylmethane	101-77-9

The International Isocyanate Institute (III) project identification number, **E-CE-95**, has been marked as part of the title of this report. Please refer to this III identification number in any communication regarding this study. **The enclosed report does not contain any Confidential Business Information.**

This study is sponsored by the International Isocyanate Institute on behalf of the following:

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Very truly yours,

Richard K. Rigger

R. K. Rigger
Managing Director

RKR/sha

REPORT

DETERMINATION OF THE EFFECT OF TDI, TDA, MDI AND MDA ON THE EMERGENCE AND GROWTH OF THE PLANT SPECIES AVENA SATIVA AND LACTUCA SATIVA ACCORDING TO OECD GUIDELINE NO.208

N.VAN DER HOEVAN, P ROZA AND L.HENZEN

TNO Institute of Environmental Sciences,
Delft, The Netherlands.

See III Report 11025 for a further terrestrial study (Earthworm)

E-CE-95

11024
93.01.25

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" SEE APPENDED (pp7-8)"

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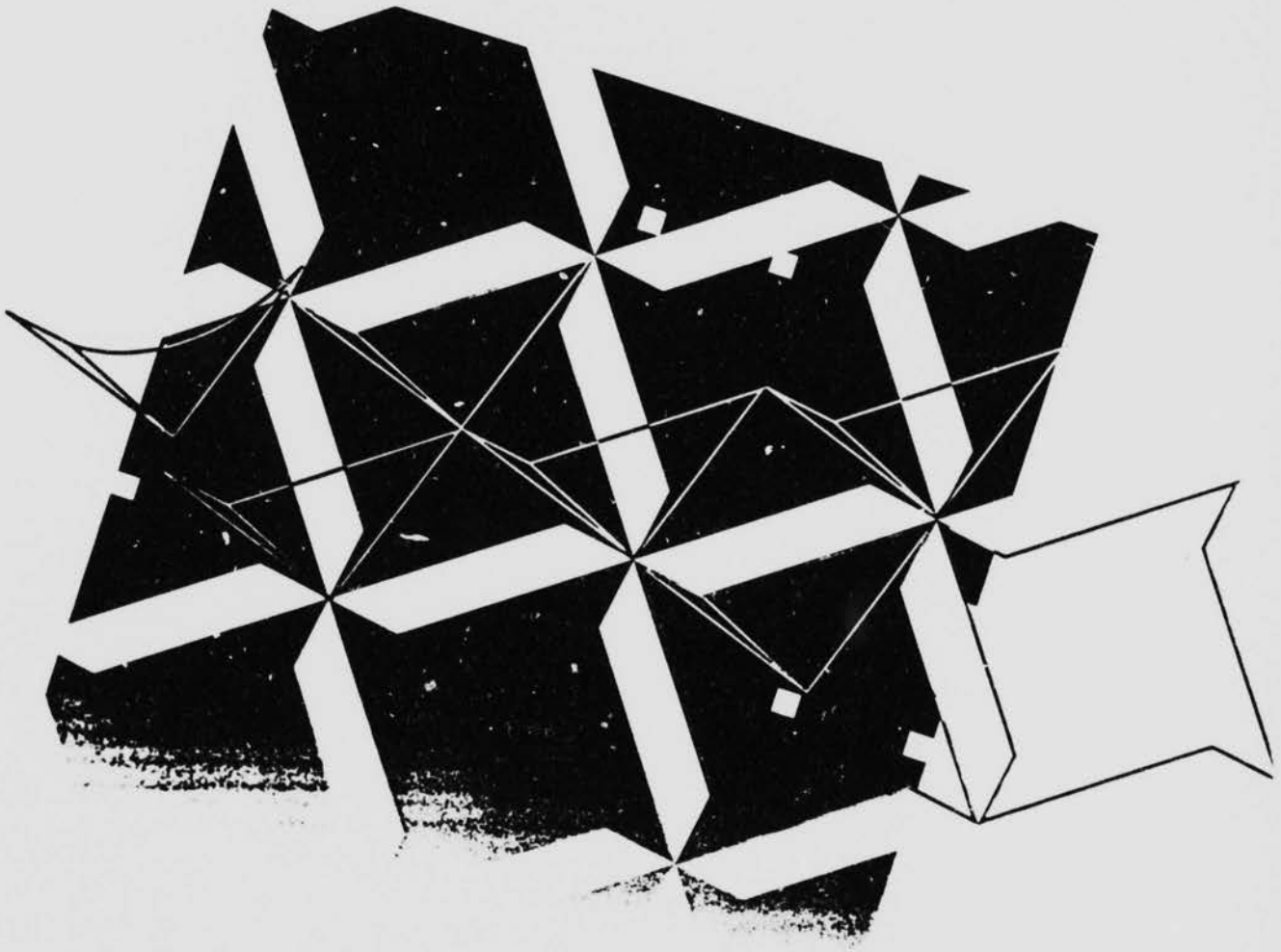

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R 92/201

TNO-report

Determination of the effect of TDI, TDA, MDI and MDA on the emergence and growth of the plant species *Avena sativa* and *Lactuca sativa* according to OECD Guideline no. 208. Project E-CE-95

Author : Dr N. van der Hoeven
P. Roza
L. Henzen

TNO Study no. : IMW-91-0032-02/03
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U.K.

Approved by: Dr R.J. Dortland

Number of pages: 44

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CONFIDENTIALITY STATEMENT

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GLP COMPLIANCE STATEMENT

'I, the undersigned, hereby declare that the work to which this report refers was performed under my supervision according to the procedure herein described. To the best of my knowledge this report provides an accurate record of the results obtained. The study was carried out in compliance with the OECD code of Good Laboratory Practice. Characterization and verification of the test substance identity and properties is, however, the responsibility of the sponsor.'

Dr N. van der Hoeven
Study Director

Date: 24 November 1992.

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Report no.: R92/201
Study no. : IMW-91-0032-02
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IMW-91-0036-02
IMW-91-0036-03

QUALITY ASSURANCE STATEMENT

STUDY TITLE: Determination of the effect of TDI, TDA, MDI and MDA on the emergence and growth of the plant species Avena sativa and Lactuca sativa according to OECD Guideline no.208
Project E-CE-95

REPORT DATE: November 24, 1992

The following inspections relevant to this study have been carried out by the Quality Assurance Unit of the TNO Institute of Environmental Sciences (IMW), P. O. Box 6011, 2600 JA Delft, the Netherlands.

Type of inspection	Date and number of inspections	Date of report to Study Director
protocol:	April 19, 1991 (1)	April 19, 1991
experimental phase:	March 12, 1992 (2)	March 13, 1992
	April 6, 1992 (1)	April 6, 1992
	April 13, 1992 (1)	April 13, 1992
	April 23, 1992 (1)	April 23, 1992
report audit:	October 27, 1992 (1)	October 30, 1992

Any serious deviations were reported to management at the same time as the report to the study director; any other, less serious deviations were reported to management upon receipt of the reply from the Study Director.

I, the undersigned, hereby declare that to the best of my knowledge this report provides an accurate record of the results obtained in this study.

M. A. Bikker
Quality Assurance Officer

Date: December 10, 1992

SUMMARY AND CONCLUSIONS

The toxicity of the substances toluene diisocyanate 80/20 (TDI), toluene diamine 80/20 (TDA), diphenyl-methane-diisocyanate (MDI) and 4,4'-diaminodi-phenylmethane, laboratory product (MDA) to the plant species *Avena sativa* (oats) and *Lactuca sativa* (lettuce) were tested in accordance with the OECD Guideline no. 208 (ref. 1) and the draft EC Guideline (ref. 2) and the OECD principles of Good Laboratory Practice (ref. 3). The toxic endpoints were emergence of the seedlings, growth (wet-weight at the end of the test), survival of seedlings and visual appearance of the plants.

For each concentration 4 x 5 plants were grown in a mixture of agricultural soil and coarse sand, supplemented with potassium and phosphorus. The plants were exposed for 17 days; emergence of the seedlings in the controls occurred within 3 days. After emergence the plants were further exposed for a growth period of at least 14 days.

The concentrations of the test substances are expressed in $mg.kg^{-1}$ of the dry soil mixture. These concentrations refer to the test substance as supplied by the sponsor.

Range-finding tests were performed with the four test substances in concentrations of 0, 10, 100 and 1000 $mg.kg^{-1}$. Based on the results of these range-finding tests the concentrations of the final tests were chosen. In the final tests, the dosed concentrations were:

TDI:	*Avena sativa*:	0 and 1000 $mg.kg^{-1}$
	Lactuca sativa:	0, 320 and 1000 $mg.kg^{-1}$
TDA:	both species:	0, 10, 32, 100, 320 and 1000 $mg.kg^{-1}$
MDI:	both species:	0 and 1000 $mg.kg^{-1}$
MDA:	*Avena sativa*:	0, 10 ,32, 100, 320 and 1000 $mg.kg^{-1}$
	Lactuca sativa:	0, 3.2, 10, 32, 100 and 320 $mg.kg^{-1}$

The observed and estimated effect concentrations for the species *Avena sativa* (As) and *Lactuca sativa* (Ls), expressed in mg of test substance per kg dry soil, were:

	TDI		TDA		MDI		MDA	
	As	Ls	As	Ls	As	Ls	As	Ls
NOEC emergence :	≥1000	≥1000	320	100	≥1000	≥1000	320	100
NOEC survival (14 days) :	≥1000	≥1000	≥1000	320	≥1000	≥1000	≥1000	≥1000
NOEC growth (14 days) :	≥1000	≥1000	320	100	≥1000	≥1000	100	10
EC50 growth (14 days) :	>1000	>1000	>320 <1000	>320 <1000	>1000	>1000	353	128

No effects on emergence, survival or wet-weight, were observed for the two diisocyanates (TDI and MDI) after 17 days (a three day germination and emergence period and a 14 days growth period) exposure to the highest test concentration, i.e. 1000 $mg.kg^{-1}$ of dry soil.
The two diamines (TDA and MDA) appeared to be more toxic than the corresponding diisocyanates. TDA affected survival of one of the plant species, *Lactuca sativa*, during the first 14 days after emergence. For TDA the toxic endpoints growth and emergence are equally sensitive, for MDA growth is a more sensitive toxic endpoint than emergence. Comparing the most sensitive toxic endpoint for each of the two diamines, MDA was observed to be more toxic than TDA.
The dicotyledonous plant *Lactuca sativa* was more sensitive to TDA and MDA than the monocotyledonous *Avena sativa*.

The environmental conditions during the experiments were as follows:

Temperature : 19 to 25°C
Moisture content : about 25% (based on dry constituents)
pH at start : between 7.5 and 7.7
pH at end : between 7.7 and 7.8
Light-dark regime : 16 hours light, 8 hours dark
Light intensity : 6000 to 8000 lux

1. INTRODUCTION

The toxicity of the substances toluene diisocyanate 80/20 (TDI), toluene diamine 80/20 (TDA), diphenyl-methane-diisocyanate (MDI) and 4,4'-diaminodiphenylmethane, laboratory product (MDA) to the plant species *Avena sativa* (oats) and *Lactuca sativa* (lettuce) were determined at the request of the sponsor. The tests were carried out in conformity with the OECD Guideline no. 208 (ref. 1) and the Draft EC Guideline (ref. 2) and the OECD principles of Good Laboratory Practice (ref. 3). The duration of the tests was 17 days. The test substances were supplied by the sponsor.

The four test substances were tested separately.

For each test substance the objectives of the studies were to determine in case effects could be observed at concentrations at or below 1000 mg.kg^{-1} of dry soil:

- the maximum concentration tested producing no inhibition of emergence of seedlings, and the maximum concentration tested producing no mortality, growth inhibition or any visual abnormalities during a 14 days growth period.
- the 14 days EC50(growth) of the test substance, i.e. the concentration which reduces the wet-weight of the plants after a 14 days growth period to 50% of the wet-weight of the plants in the control medium.
- the 17 days EC50(emergence) of the test substance, i.e. the concentration which reduces the emergence of the seedlings to 50% of the emergence in the control medium.

Otherwise, the objective of the studies were to determine in a limit test whether no effects could be found at a concentration of 1000 mg.kg^{-1} of dry soil.

The effects of concentrations higher than 1000 mg of test substance per kg of dry soil were not investigated.

Relevant dates for the tests were:

TDI: Protocol (GLP 91/058) signed by the Study Director on:
April 19, 1991
Amendment No. 1 to this protocol signed by the Study Director on:
October 4, 1991
Period of range finding tests: February 13, 1992 to March 2, 1992
Period of Final tests, *A. sativa*: March 20, 1992 to April 6, 1992
L. sativa: April 6, 1992 to April 23, 1992

TDA: Protocol (GLP 91/059) signed by the Study Director on:
April 19, 1991
Amendment No. 1 to this protocol signed by the Study Director on:
October 4, 1991
Period of range finding tests: February 21, 1992 to March 9, 1992
Period of Final tests: March 12, 1992 to March 29, 1992

MDI: Protocol (GLP9 1/060) signed by the Study Director on:
April 19, 1991
Amendment No. 1 to this protocol signed by the Study Director on:
October 4, 1991
Period of range finding tests: March 11, 1992 to March 27, 1992
Period of Final tests: April 6, 1992 to April 23, 1992

MDA: Protocol (GLP 91/062) signed by the Study Director on:
April 19, 1991
Amendment No. 1 to this protocol signed by the Study Director on:
October 4, 1991
Period of range finding tests: February 13, 1992 to March 2, 1992
Period of Final tests: April 8, 1992 to April 25, 1992

2. MATERIALS AND METHOD

2.1 Test substance

The test substances were toluene diisocyanate 80/20 (TDI), toluene diamine 80/20 (TDA), diphenyl-methane-diisocyanate (MDI) and 4,4 -diaminodiphenylmethane, laboratory product (MDA). The test substances will be indicated in this report by the abbreviations, TDI, TDA, MDI and MDA respectively.

For these tests the following batches of test substance were used:

TDI : The batch of test substance was received on July 2, 1991 in a 1 litre aluminium screw-capped bottle. This bottle was labelled: 'Desmodur T80 Giftig 2,4/2,6-diisocyanat-toluol., Datum: 20.6.1991, Partie: 808, Tank: 6, Referenz: IMW 91/746. The test substance came in the form of a colourless to yellowish liquid. The test substance was stored at room temperature, protected from light in a closed cupboard. According to the sponsor, TDI contained 80% of the 2,4 isomer and 20% of the 2,6 isomer of toluene diisocyanate and its purity was more than 99.9%. TDI was stated to react with water and to be soluble in aceton.

TDA : The batch of test substance was received on July 2, 1991 in a 1 litre aluminium screw-capped bottle. This bottle was labelled: 'M-TDA, Giftig 2,4 u 2,6-diaminotoluol., 4.6.91, PT.12, Referenz: IMW 91/746. The test substance came in the form of a brown solid. The test substance was stored at room temperature, protected from light in a closed cupboard. According to the sponsor the batch contained more than 99% active ingredient, i.e. toluene diamine. The water solubility of TDA was stated to be about 100 $g.l^{-1}$.

MDI : The batch of test substance was received on February 24, 1992 in a 1 litre aluminium screw-capped bottle. This bottle was labelled: '4,4' diphenylmethan-diisocyanat, isomere/homologe, harmful, Bayer AG'. The test substance came in the form of a dark-brown liquid. The test substance was stored at room temperature, protected from light in a closed cupboard. According to the sponsor the active ingredients of MDA were diphenyl-methane-diisocyanate (isomers and homologous) and consisted of 40-50% of the 4,4'-isomer, 2-4% of the 2,4'-isomer and 40-60% of 3-ring isomers. MDI contained traces of phenylisocyanate and

monochlorbenzene as impurities. MDI was stated to react with water, forming urea and CO_2 and to be soluble in aceton.

MDA : The batch of test substance was received on January 20, 1992 in a 1 litre square glass bottle with a blue screw-cap. This bottle was labelled: 'Referenz IMW 91/746, 4,4'-diamino-diphenylmethan, BMC 200/10: MDA 100 dest'. The test substance came in the form of a colourless to light yellow solid lump. The test substance was stored at room temperature, protected from light in a closed cupboard. According to the sponsor its purity was more than 99.5% of the active ingredient, 4,4'-diaminodiphenylmethane (laboratory product). MDA contained traces of 2,4'-diaminodiphenylmethane and higher molecular weight oligomers as impurities. MDA is stated to be practically insoluble in water and to be soluble in aceton.

The composition and properties of the four test substances as specified by the sponsor are recorded in Annex A.

2.2 Test organism

The test organisms were the plant species *Avena sativa* L. (oats) and *Lactuca sativa* L. (lettuce). The seeds of *Avena sativa* (oats) were obtained from CEBECO (Roterdam, the Netherlands) on June 13, 1991. The seeds of *Lactuca sativa* L. 'Ravel RZ' (Lettuce) were obtained from Rijk Zwaan (De Lier, the Netherlands) on February 18, 1991.

The seeds of *Avena sativa* were sown at a depth of about 1 cm, the top of the seeds upwards and were covered with soil. The final adjustment of the moisture content of the soil in the test vessels was carried out after sowing.

The seeds of *Lactuca sativa* were sown on top of the soil. No water was added after the sowing of *Lactuca sativa*.

2.3 Description and preparation of semi-natural soil

The soil used was a mixture (1:1) of agricultural soil from an orchard in Heerewaarden (the Netherlands) and coarse sand (grain size 500 to 1000 µm). A quantity of 1.66 g K_2HPO_4 was added per kg of dry soil mixture to assure good growth conditions. The organic carbon content of the soil-sand mixture was 1.1%. The pH (KCl) of this soil was between 7.5 and 7.7. This is slightly higher than the pH range of 5.0 to 7.5 prescribed in the OECD (ref. 1) and EC (ref. 2) guidelines. It is not expected that this slight deviation in pH influences the results of these tests. The composition of the agricultural soil is given in annex C.

For test substances which were sufficiently soluble to be dosed directly in water to the relatively dry soil-sand mixture, the agricultural soil, the sand and the appropriate amount of test substance dissolved in the amount of water necessary to obtain the desired moisture content in the soil were mixed per concentration. Mixing was done in a polyethylene bowl using a household mixer. However, only TDA could be dosed in this manner.

Test substances which could be dissolved in acetone, were prepared by another method. The coarse sand was separately coated with the test substance for each test substance concentration. The coating was performed in a glass cylinder, the appropriate amount of sand (900 g) being soaked with a solution of the test substance in acetone. The evaporation of the acetone was speeded up by blowing N_2 through the sand from below. The coated sand was mixed in a stainless steel bowl with the agricultural soil and the appropriate amount of water using a hand mixer. Soils with TDI, MDI and MDA were prepared in this way.

2.4 Test method

The tests were conducted in accordance with the OECD Guideline no. 208 (ref. 1) and the Draft EC Guideline (ref. 2). Range-finding tests were performed with the four test substances and the two test species to determine the test concentration in the final test. In the range-finding test with TDI and MDI no effects were observed in either of the test species at a concentration of 1000 $mg.kg^{-1}$ of dry soil. Therefore, in the final tests MDI was tested in a limit test with both plant species and limit tests of TDI with both plant species were started.

However, in the limit test with TDI and *Lactuca sativa* the soil appeared to be thightly set and very wet at 1000 $mg.kg^{-1}$ of dry soil. This might influence the seed emergence and growth. Therefore, this test was i terrupted and a new final test of TDI with *Lactuca sativa* was started with an extra test concentration of 320 $mg.kg^{-1}$ of dry soil.
The preparation of the test medium is described for each test substance separately.
The moisture content recorded is always based on dry constituents.

2.4.1 Preparation of test medium with TDI

TDI was tested in a limit test, i.e. only controls and a test substance concentration of 1000 $mg.kg^{-1}$ dry soil were tested for the plant species *Avena sativa*. *Lactuca sativa* was also tested with 320 mg TDI per kg dry soil. The tests with *A. sativa* and *L. sativa* were not performed simultaneously. Different batches of soil were prepared per concentration and per plant species.

For the test with *Avena sativa* a quantity of 4.58 g of TDI was accurately weighed and dissolved in 254.4 ml of acetone. From this solution, 100 ml was added to 900 g of the coarse sand. The sand was coated as described in section 2.3. Once coated, the sand was mixed with 1016 g of agricultural soil (moisture content 12.8%; 900 g dry agricultural soil, 116 g water) to reach a test concentration of 1000 mg TDI per kg of dry soil. Controls were prepared in a similar manner by adding 100 ml of pure acetone to 900 g coarse sand. A quantity of 2.99 g K_2HPO_4 was added to this soil mixture and was thoroughly mixed with 150 ml of demineralized water.

A quantity of 367 g of the thus prepared soil was placed in each test vessel, a plastic cup (320 g dry soil, 47 g water), and was filled up to 400 g with demineralized water. Five cups containing 1000 mg of TDI per kg of dry soil and five cups containing control soil were prepared in this way

For the test with *Lactuca sativa*, a quantity of 4.51 g of TDI was accurately weighed and dissolved in 250 ml of acetone. A dilution of this stock solution was made by diluting 32 ml of this solution to 100 ml with acetone. From these solutions, 100 ml was added to 900 g of the coarse sand. The sand was coated as described in section 2.3. Once coated, the sand was mixed with 1014 g of agricultural soil (moisture content 12.7%; 900 g dry agricultural soil, 114 g water) to reach test concentrations of 320 and 1000 mg TDI per kg

of dry soil. Controls were prepared in a similar manner by adding 100 ml of pure acetone to 900 g coarse sand. A quantity of 2.99 g K_2HPO_4 was added to this soil mixture and was thoroughly mixed with 150 ml of demineralized water.

A quantity of 367 g of the thus prepared soil was placed in each test vessel, a plastic cup (320 g dry soil, 47 g water), and was filled up to 400 g with demineralized water. Five cups containing 320 and 1000 mg of TDI per kg of dry soil and five cups containing control soil were prepared in this way.

2.4.2 Preparation of test medium with TDA

A quantity of 6.10 g of TDA was accurately weighed and dissolved in demineralized water to make up a total volume of 508.3 ml. Aliquots of 1.5, 4.8, 15 and 48 ml of this stock solution were made up to 150 ml with demineralized water. These solutions and 150 ml of the stock solution were each added to 1920 g of the soil-sand mixture (moisture content 6.5%; 1800 g of dry soil mixture (agricultural soil:coarse sand 1:1 and 2.99 g K_2HPO_4), 116 g water) to reach test concentrations of 10, 32, 100, 320 and 1000 mg TDA per kg of dry soil. Controls were prepared in a similar manner by adding 150 ml of demineralized water to 1920 g soil-sand mixture. This mixture was thoroughly mixed.

A quantity of 367 g of the thus prepared soil was done in each test vessel, a plastic cup (320 g dry soil, 47 g water), and the cups were filled up to 400 g with demineralized water. Five cups containing 10, 32, 100, 320 and 1000 mg of TDA per kg of dry soil and five cups containing control soil were prepared in this way.

2.4.3 Preparation of test medium with MDI

MDI was tested in a limit test, i.e. only controls and a test substance concentration of 1000 $mg.kg^{-1}$ dry soil were tested with both plant species. A quantity of 4.59 g of MDI was accurately weighed and dissolved in 255 ml of acetone. From this solution, 100 ml was added to 900 g of the coarse sand. The sand was coated as described in section 2.3. Once coated, the sand was mixed with 1014 g of agricultural soil (moisture content 12.7%; 900 g dry agricultural soil, 114 g water) to reach test concentrations of 1000 mg MDI per kg of dry soil. Controls were prepared in a similar manner by adding 100 ml of pure acetone to

900 g coarse sand. A quantity of 2.99 g K_2HPO_4 was added to this soil mixture and was thoroughly mixed with 150 ml of demineralized water.

A quantity of 367 g of the thus prepared soil was done in each test vessel, a plastic cup (320 g dry soil, 47 g water), and was filled up to 400 g with demineralized water. Five cups containing 1000 mg of MDI per kg of dry soil and five cups containing control soil were prepared in this way.

2.4.4 Preparation of test medium with MDA

A quantity of 9.13 g of MDA was accurately weighed and dissolved in 507.2 ml acetone. Aliquots of 0.8, 2.5, 8.0, 25 and 80 ml of this stock solution were made up with 250 ml of acetone. From these solutions, 100 ml was added to 900 g of the coarse sand. The sand was coated as described in section 2.3. Once coated, the sand was mixed with 1014 g of agricultural soil (moisture content 12.7%, 900 g dry agricultural soil, 114 g water) to reach test concentrations of 3.2, 10, 32, 100, 320 and 1000 mg MDA per kg of dry soil. Controls were prepared in a similar manner by adding 100 ml of pure acetone to 900 g coarse sand. A quantity of 2.99 g K_2HPO_4 was added to this soil mixture and was thoroughly mixed with 150 ml of demineralized water.

A quantity of 367 g of the thus prepared soil was done in each test vessel, a plastic cup (320 g dry soil, 47 g water), and was filled up to 400 g with demineralized water. Five cups containing 3.2, 10, 32, 100, 320 and 1000 mg of MDA per kg of dry soil and five cups containing control soil were prepared in this way.

2.4.5 Test conditions and measurements

A series of five tests cups were prepared per test substance concentration and per control. Seeds were sown in each of four of these; the fifth was used for pH measurement.

The tests were carried out in a thermostatically controlled room under a light-dark regime of 16 hours light and 8 hours dark. The light intensity during the light period was about 6500 lux (between 6000 and 8000 lux). Near the plants the temperature varied between 19°C (dark period) and 25°C (light period). The test vessels were circular plastic cups

(food quality) with a maximum diameter of about 9.3 cm and a height of about 6.0 cm. The cups were filled to about 2/3 with soil. Water could not drain from the cups; the cups were only open at the top side.

About 10 seeds were sown in each of the cups and covered with a glass plate. The space between the glass plate and the top of the soil was about 2 cm. It was removed after the seeds had germinated and before they grew tall enough to touch it. All emergence of seedlings was recorded. Five seedling were left to grow, the others were removed. These five remaining plants were chosen from the first group of germinated plants so that they did not grow too closely together. Preferably, only healthy looking individuals were chosen.

At the start of the tests, the soil had a moisture content of about 25% (based on dry constituents). After the glass plates were removed from the cups, they were weighed daily, and demineralized water was added to the soil to bring the moisture content back to the initial level (the weight of the plants in the cup was ignored and to compensate for the weight of the plastic cup, the balance was tared using another plastic cup)

At the start of each test the pH values in the control soils were determined (to measure the pH, 50 g of soil was added to 100 ml of 0.1 M KCl and the pH of the supernatant determined after one hour. The pH was measured in threefold. The mean of these values is given). The pH at the start of the test with TDI (*A. sativa* and *L. sativa* separately), TDA, MDI and MDA were: 7.6 (*A. sativa*), 7.7 (*L. sativa*), 7.5, 7.7 and 7.7 respectively.

The tests lasted for 17 days, including 3 days (at most) until emergence of the seedlings from the soil in the controls and at least 14 days after emergence. Emergence of the seedlings was recorded. Several times a week the visual appearance of the plants was assessed in comparison to the controls. Mortality of the plants was recorded. At the end of each test (at least 14 days after emergence of the seedlings in the control) the shoots of all surviving plants were harvested and immediately weighed individually. *Avena sativa* was harvested by cutting of the shoots immediately above the thickening at the place of the seed envelope. *Lactuca sativa* was harvested by cutting of the shoots immediately above the first root.

At the end of each test the pH of the soil in which the plants were grown was determined for both species separately. No differences in pH values were found between both species.

The mean pH values in the controls at the end of the test all felt within the range of 7.7 to 7.8.

2.5 Treatment of the results

2.5.1 NOEC values

The 'no observed effect concentrations' (NOEC values) are the highest concentrations tested showing no effects (defined below) throughout the exposure period. The NOEC values were estimated by comparing emergence, mortality, wet-weight at the end of the growth period and visual appearance of the exposed seeds and plants with those of the control seeds and plants.

To determine the NOEC for emergence and mortality, the dates of emergence of seedlings and the mortality dates of each concentration were compared pair-wise with those in the control using a binomial test (2 x 2 contingency table). A significance level of 5% was used. Effects on emergence could both come in the form of postponement and of complete prevention.

To determine the NOEC for wet-weight after the growth period, a multiple comparison was made between the wet-weight of each plant at each concentration and the wet-weight of the plants in the control using a two-tailed Dunnett test. It was assumed that the plants in each cup were growing independently of each other. A significance level of 5% was used.

The NOEC was determined as follows:

- At the NOEC no significant differences with the controls were observed.
- At the first higher test concentration (LOEC; lowest observed effect concentration) a significant difference with the controls was observed.

- At all higher concentrations tested, the differences with the controls were either also significant or larger than those at the LOEC.

The NOEC for physical appearance of the plants was not determined statistically.

2.5.2 EC50 (growth) values

The effect of the test substance on growth of the plants is expressed by a quantity denoted as the EC50(growth) (= Effect Concentration, 50% on growth), i.e. the exposure concentration of the test substance which would reduce the weight of the plants after a given growth period to 50% of the weight of the plants in the control medium.

The tests with TDI and MDI, however, were not designed to calculate an EC50(growth) value (because no effect was expected at 1000 $mg.kg^{-1}$; only that one concentration, and in one case also 320 $mg.kg^{-1}$, was tested).

For TDA, the concentration: wet-weight relationship was not suitable for calculations (the number of concentrations causing intermediate effects were too small). The EC50(growth) value is therefore only given as being between the highest test concentrations leading to a wet-weight of more than 50% of the control and the lowest test concentration leading to a wet-weight of less than 50% of the control.

For MDA, the EC50(growth) values were calculated using an iterative maximum likelihood estimation procedure which assumes a relationship between wet-weight (W) at the end of the exposure period and exposure concentration (C) of the form

$$W(C) = W(0)/[1+ (C/EC50)^{g}]$$

where g is a slope parameter, i.e. the larger g becomes, the steeper the concentration-effect curve. A normally distributed error in the wet-weight was assumed.

2.5.3 EC50 (emergence) values

The effect of the test substance on emergence of seedlings is expressed by a quantity denoted as the EC50(emergence) (= Effect Concentration, 50% on emergence), i.e. the exposure concentration of the test substance which would reduce the emergence of the seedlings after a given exposure period to 50% of the emergence of seedlings in the control medium.

The tests with TDI and MDI, however, were not designed to calculate an EC50(emergence) value (because no effect was expected at 1000 $mg.kg^{-1}$; only that one concentration, and in one case also 320 $mg.kg^{-1}$, was tested).

For TDA and *Avena sativa*, the 17d EC50(emergence) value was calculated using an iterative maximum likelihood estimation procedure which assumes a relationship between % emergence (E) at the end of the exposure period and exposure concentration (C) of the form

$$E(C) = 100/[1+ (C/EC50)^{a}]$$

where a is a slope parameter, i.e. the larger a becomes, the steeper the concentration-effect curve. Emergence of the seedlings was assumed to be independent. This method is described by Kooijman (ref. 4) for survival data.

For MDA, the concentration emergence relationship was not suitable for calculation of the EC50. For both plant species the % emergence at the end of the test was more than 50%, even in the highest test concentration.

3. RESULTS

The results of the tests, expressed as NOEC and EC50 values, are presented in table 1.
The number of seeds sown, the number of emerged seedlings and the plant mortality are listed in annex B, tables B1.1 to B1.4 together with the mean wet-weight of the plants at the end of the tests and the visual estimated condition of the plants.
The individual wet-weights of the plants are listed in annex B, tables B2.1 to B2.4.
The plants were exposed for 17 days; emergence of the seedlings in the controls occurred within 3 days.

Table 1. *Results of the tests with TDI, TDA, MDI and MDA and the plant species Avena sativa (As) and Lactuca sativa (Ls).*

Parameter	Effect	Plant species	Nominal concentration (mg.kg^{-1} dry soil)			
			TDI	TDA	MDI	MDA
NOEC	emergence	As	≥1000 [1]	320 [8]	≥1000 [1]	320
		Ls	≥1000 [1]	100	≥1000 [1]	100
NOEC	mortality of seedlings	As	≥1000 [1]	≥1000 [1]	≥1000 [1]	≥1000 [1]
		Ls	≥1000 [1]	320	≥1000 [1]	≥1000 [1]
NOEC	appearance	As	≥1000 [1]	320 [2]	≥1000 [1]	100 [3]
		Ls	≥1000 [1]	100 [3]	≥1000 [1]	10 [4]
NOEC	growth (wet-weight)	As	≥1000 [1]	320	≥1000 [1]	100
		Ls	≥1000 [1]	100	≥1000 [1]	10
EC50	growth (wet-weight)	As	>1000 [5]	>320; <1000 [1]	>1000 [5]	353 [6]
		Ls	>1000 [5]	>320; <1000 [1]	>1000 [5]	128 [7]

1) Highest concentration tested.
2) Observed effect at 1000 mg.kg^{-1}: Plants were smaller than in control and dark-green. One of the plants seemed to be death half-way the test, but at the end of the test this plant was found to be still alive.
3) Observed effect at 320 mg.kg^{-1}: Plants were smaller than in control.
4) Observed effect at 32 mg.kg^{-1}: Plants were less regular in size than in control, at 100 mg.kg^{-1} the plants were smaller than in the control.
5) EC50 value could not be determined, since even at the highest concentration tested no effects were observed on growth.
6) 95% confidence interval 329-379 mg.kg^{-1} dry soil.
7) 95% confidence interval 116-142 mg.kg^{-1} dry soil.
8) At 320 mg.kg^{-1} emergence was observed of only 36 of the 40 seeds. This reduced emergence, however, did not deviate significantly (5% level) from the control emergence.

No effects on the emergence of seedlings, survival, appearance or growth were observed in either of the two plant species tested after 17 days exposure to the highest test concentration for the two diisocyanates (TDI and MDI) (1000 mg.kg^{-1} dry soil). Since both diiso-

tration for the two diisocyanates (TDI and MDI) (1000 mg.kg^{-1} dry soil). Since both diisocyanates react with water, the absence of any effects may be due to the disappearance of the diisocyanates from the test medium.

The two diamines (TDA and MDA) appeared to be more toxic than the corresponding diisocyanates. With respect to growth, the most sensitive toxic endpoint in this test, MDA was more toxic than TDA to both *Avena sativa* and *Lactuca sativa*.
TDA affected the survival of *Lactuca sativa* after emergence at 1000 mg.kg^{-1} dry soil. No effects from MDA on plant survival were observed.
Both TDA and MDA affected the emergence of the seedlings. The highest test concentration of TDA reduced seedling emergence before the end of the test of *Avena sativa* to less than 50%. The 17d EC50 (emergence) of *Avena sativa* for TDA is 904 mg.kg^{-1} of dry soil (95% confidence interval 700-1170 mg.kg^{-1}).
For TDA, emergence of seedlings and growth proved to be equally sensitive toxic endpoints. MDA affects growth more severely than seedling emergence. Effects on seedling emergence appeared for both TDA and MDA in two forms: reduction in the number of seedlings emerging and postponement of emergence. Theoretically, a reduction in wet-weight at the end of the test can be caused by delayed germination. For *Lactuca sativa*, germination of the seeds and emergence of the seedlings occur simultaneously, since the seeds of *L. sativa* are placed on top of the soil and not in it. With *Avena sativa*, delayed seedling emergence can point to delayed germination of the seeds. TDA delays seedling emergence, and therefore germination of *Lactuca sativa* and may be of *Avena sativa*, by at most 4 days at concentrations of 1000 and 320 mg.kg^{-1} respectively. The reductions in wet-weight induced by these concentrations of TDI are too large to be explained by a delay in germination (wet-weight at these concentrations less than 20% and 10% of the wet-weights in the controls for *A. sativa* and *L. sativa* respectively). For MDA, effects on wet-weight were already observed at concentrations at which no effects on seedling emergence were observed. Therefore, for both diamines and both plant species, the effect on wet-weight is an effect on growth and not an indirect effect as a result of delayed germination.
For both diamines (TDA and MDA) the dicotyledonous plant *Lactuca sativa* (lettuce) was more sensitive than the monocotyledonous *Avena sativa* (oats).

4. REFERENCES

1) OECD Guideline for testing of chemicals
no. 208 'Terrestrial plants, growth test'
Organization for Economic Co-operation and Development, Paris (1984).

2) Methods for the determination of ecotoxicity, level 1: Higher plants (C(L1)3)
EC Directive 79/831, annex V, part C, 3th draft, 1986.

3) Good Laboratory Practice in the testing of chemicals
Organization for Economic Co-operation and Development, Paris (1982).

4) Kooijman, S.A.L.M. (1981).
Parametric analyses of mortality rates in bioassays.
Water Res. 15, 107-119.

5. RETENTION OF RECORDS AND SAMPLES

All the data generated and all other information relevant to the quality and integrity of these studies have been filed under the study references IMW-91-0032-02/03 (TDI), IMW-91-0033-02/03 (TDA), IMW91-0034-02/03 (MDI) and IMW-91-0036-02/03 (MDA) in the archives of the TNO Institute of Environmental Sciences, Schoemakerstraat 97, 2628 VK Delft, The Netherlands. These records will be retained for a period of at least ten years after the cover date of this report.

Samples of the test substances have been deposited under the sample references IMW-91-0032-A (TDI), IMW-91-0033-A (TDA), IMW-91-0034-A (MDI) and IMW-91-0036-A (MDA) in the sample archives of the TNO Institute of Environmental Sciences at the same address; these samples will be stored for a period of at least ten years.

6. DEVIATIONS FROM THE PROTOCOL

The pH values were not determined at the highest concentration of the test substances to prevent the risk of the contamination of the pH electrode. The OECD Guideline 208 (ref. 1) and the Draft EC Guideline (ref. 2) do not prescribe these measurements.

TDA was dosed by dissolving in water, and not in acetone.

The acetone in which the three of the test substances were dosed (TDI, MDI and MDA) was mixed with the coarse sand, and the evaporation of the acetone was speeded up by blowing N_2 through the sand from below.

For the tests with TDI, MDI and MDA, controls were only used in which the soil was treated with the solvent acetone, in a similar manner to the soils with the test substance. This is in accordance with the OECD Guideline 208 (ref. 1) and the Draft EC Guideline (ref. 2).

The laboratory product MDA was given TNO code SIE. However, until February 28, 1992 this test substance was errorously given TNO code SID. Since the test substance which was originally allocated this code (MDA, commercial product), was removed from the TNO test substance list and furthermore was only slightly different from SIE, no consequences can be expected from this mistake.

At the end of the dark period the temperature near the plants dropped to about 19°C.
The temperature was not measured every day during the experiment, but sampled periodically.

In the protocols, the test substances TDI and TDA were indicated as TDI 80/20 and TDA 80/20 respectively. The abbreviation MDA for the test substance 4,4'-diaminodiphenylmethane, laboratory product, was not used in the protocol.

Mr A. van Mullen assisted in some of the tests.

ANNEX A COMPOSITION AND PROPERTIES OF TDI, TDA, MDI AND MDA

A1 Composition and properties of TDI

DIVISION OF TECHNOLOGY FOR SOCIETY TNO
DEPARTMENT OF BIOLOGY

Form no. : MTB/EG/005
For : Characterization of the test substance
Belonging to : Standard operating procedure MTB/PG/003

Test substance name or code to be used in report: Toluene Diisocyanate 80/20
TDI 80/20

Storage conditions:

Storage temperature: ~~freezer~~ | ~~refrigerator~~ | room temperature | ~~special (specify)~~ _____ *

Photostability: ~~dark~~ | protect from light *

Expiry date: 6 months from sample date = 20-1-

* delete where applicable

Characterization:

Physical appearance: colourless to yellowish liquid at room temperature

Boiling point: 247 °C at 760 mm Hg Melting point: 12,5-13,5 °C Density: 1,21 g/cm³

Batch no.: R08 Quantity submitted: 1 kg

Active ingredient: Toluene Diisocyanate (80 % 2,4 isomer/20 % 2,6 isomer)

Carrier, solvent or diluting agent: ./.

Percentage content of active ingredient: >99,9

Nature and quantity of impurities: Chlorine containing aromatic substances

Solvent	Solubility	Maximum storage time of solution
water	TDI reacts with water	
acetone	Yes	solution should be freshly prepared
methanol	} TDI reacts with alcohols	
ethanol		
dimethylsulphoxide	TDI reacts with DMSO	

Information on toxicity (acute toxicity, oral-, dermal or inhalation toxicity, skin- and eye irritation, sensitization, carcinogenicity , mutagenicity, etc.):

DIN Safety Data Sheet
Bayer 043412/01
31 October 1990

Is the test substance explosive, inflamable, corrosive:

Other special handling instructions:

Form completed by: Signature: Date:

TNO study no.: ~~M T B~~ IMW 91-0032-03

2/900601

RA92201IMWja

A2 Composition and properties of TDA

DIVISION OF TECHNOLOGY FOR SOCIETY TNO
DEPARTMENT OF BIOLOGY

Form no. : MTB/EG/003
For : Characterization of the test substance
Belonging to : Standard operating procedure MTB/PG/003

Test substance name or code to be used in report: Toluene Diamine 80/20
TDA 80/20

Storage conditions:

Storage temperature: ~~freezer~~ | ~~refrigerator~~ | room temperature | ~~specify (specify)~~ _____ *

Photostability: ~~good~~ | protect from light *

Expiry date: 6 months from sample date

* delete where applicable

Characterization:

Physical appearance: brown solid

Boiling point ca 288 °C at 760 mm Hg Melting point: ca 100 °C Density: ca 1 g/cm³

Batch no.: 13 Quantity submitted: 1 kg

Active ingredient: Toluene Diamine

Carrier, solvent or diluting agent: -/-

Percentage content of active ingredient: >99 %

Nature and quantity of impurities: high boiling residues

Solvent	Solubility	Maximum storage time of solution
water	yes (100 g/l)	solution should be freshly
acetone	"	prepared each time
methanol	"	
ethanol	"	
dimethylsulphoxide	not tested	

Information on toxicity (acute toxicity, oral-, dermal or inhalation toxicity, skin- and eye irritation, sensitization, carcinogenicity, mutagenicity, etc.):

Is the test substance explosive, inflamable, corrosive: NIN Safety Sheet Bayer 011405/05 5 December 1990

Other special handling instructions:

Form completed by: Signature: Date:

TNO study no: ~~MTB~~ IMW 91-0033-01

2900601

RA92201IMWja

A3 Composition and properties of MDI

DIVISION OF TECHNOLOGY FOR SOCIETY TNO
DEPARTMENT OF BIOLOGY

Form no. : MTB/EG/003
For : Characterization of the test substance
Belonging to : Standard operating procedure MTB/PG/003

Test substance name or code to be used in report: Diphenyl-methane-diisocyanate (MDI)

Storage conditions:

Storage temperature: ~~freezer~~ | ~~refrigerator~~ | room temperature | ~~special (specify)~~ _____ *

Photostability: good | ~~protect from light~~ *

Expiry date: max. 6 months storage time at 20-25°C

* delete where applicable

Characterization:

Physical appearance: dark-brown liquid

Boiling point: 250 *1 °C at 760 mm Hg Melting point: 0 *2 °C Density: 1,23 g/cm^3

Batch no: _____ Quantity submitted: _____

Active ingredient: Diphenyl-methane-diisocyanate (Isomers and Homologous)

Carrier, solvent or diluting agent: _____

Percentage content of active ingredient: 40-50% 4,4'-/2-4% 2,4'-/40-60% 3-Ring-Isomers

Nature and quantity of impurities: Traces of phenylisocyanate and monochlorbenzene

*1 decomposition *2 partial cristallisation

Solvent	Solubility	Maximum storage time of solution
water	Reaction with water yields urea and CO_2	
acetone	Yes	
methanol	Reaction with methanol yields urethane ~~and CO_2~~	
ethanol	" " ethanol " "	
dimethylsulphoxide		

Information on toxicity (acute toxicity, oral-, dermal or inhalation toxicity, skin- and eye irritation, sensitization, carcinogenicity, mutagenicity, etc.):

Safety Data Sheet
Bayer 044192/04
29 October 1990

Is the test substance explosive, inflamable, corrosive:

Other special handling instructions:

Form completed by: Signature: Date:

TNO study no.: M | T | B | | | | | | | | | | |

2:9C0601

A4 Composition and properties of MDA

DIVISION OF TECHNOLOGY FOR SOCIETY TNO
DEPARTMENT OF BIOLOGY

Form no : MTB/EG/003
For : Characterization of the test substance
Belonging to : Standard operating procedure MTB/PG/003

Test substance name or code to be used in report:
4,4'-diaminodiphenylmethane, laboratory product

Storage conditions:

Storage temperature: ~~freezer~~ | ~~refrigerator~~ | room temperature | ~~special (specify)~~ _____ *

Photostability: good | ~~protect from light~~ * Expiry date: _____

* delete where applicable

Characterization:

Physical appearance: colourless to light yellow, solid lumps

Boiling point: ~~220/~~ 230 °C at 4 mm Hg Melting point: 91-92 °C Density: ca. 1.0 gm/cm^3 ~~at 100°~~

Batch no: 8 Quantity submitted: 1

Active ingredient: 4,4'-diaminodiphenylmethane >99,5 %

Carrier solvent or diluting agent: none

Percentage content of active ingredient: >99,5 %

Nature and quantity of impurities: 2,4'-diaminodiphenylmethane (trace)
higher molecular weight oligomers (trace)

Solvent	Solubility	Maximum storage time of solution
water	practically insoluble	
acetone	soluble	
methanol	very soluble	
ethanol	soluble	
dimethylsulphoxide	(unknown)	

Information on toxicity (acute toxicity, oral-, dermal or inhalation toxicity, skin- and eye irritation, sensitization, carcinogenicity, mutagenicity, etc.)

DIN Safety Sheet
Bayer 328794/05
3 December 1990

Is the test substance explosive, inflamable, corrosive

Other special handling instructions

Form completed by Signature Date:

TNO study no: M T B [ ] – [ ]

ANNEX B INDIVIDUAL TEST DATA

Table B1 *Number of seeds sown, number of seedlings emerged on observation day, number of seedlings removed, plant mortality, plant condition and mean wet-weight of the plants at the end of the test after exposure to several concentrations of the test substances (mg.kg^{-1} dry soil).*

S: number of seeds sown on day 0
E: cumulative number of seedlings emerged (only given if at least one seedling had emerged since last observation day)
R: number of plants removed
C: condition of the plants (in codes, see legend at bottom of table)
M: cumulative number of plant mortality

Footnotes on this table are given on page 37.

Table B1.1a *Data on emergence, condition, survival and wet-weight of Avena sativa exposed to TDI.*

day	S 0	E 3	R 4	C[1] 5	C[1] 7	C[1] 11	C[1] 14	C[1] 17	M 17	weight (g) mean	s.d.	N	average weight (s.d.)[3]
conc. mg.kg^{-1}													
0	10	10	5	a	a	a	a	a	0	1.04	0.22	5	
0	10	10	5	a	a	a	a	a	0	0.97	0.11	5	0.97
0	10	10	5	a	a	a	a	a	0	0.93	0.10	5	(0.05)
0	10	10	5	a	a	a	a	a	0	0.96	0.06	5	
1000	10	10	5	b	b	b	b	b	0	0.97	0.15	5	
1000	10	10	5	b	b	b	b	b	0	0.97	0.23	5	0.99
1000	10	10	5	b	b	b	b	b	0	0.99	0.12	5	(0.02)
1000	10	10	5	b	b	b	b	b	0	1.02	0.14	5	

Emergence of seedlings was observed in the control on the third day.

The glass plate was removed on day 3.

Table B1.1b Data on emergence, condition, survival and wet-weight of Lactuca sativa exposed to TDI.

day	S	E	R	C[1)]	C[1)]	C[1)]	C[1)]	C[1)]	M	weight (g)			average weight (s.d.) [3)]
	0	4	4	9	10	15	16	17	17	mean	s.d.	N	
conc. mg.kg^{-1}													
0	10	10	5	a	a	a	a	a	0	1.40	0.15	5	
0	10	10	5	a	a	a	a	a	0	1.33	0.29	5	1.40
0	10	10	5	a	a	a	a	a	0	1.39	0.21	5	(0.07)
0	10	10	5	a	a	a	a	a	0	1.50	0.26	5	
320	10	10	5	b	b	b	b	b	0	1.39	0.20	5	
320	10	10	5	b	b	b	b	b	0	1.36	0.14	5	1.43
320	10	10	5	b	b	b	b	b	0	1.43	0.25	5	(0.08)
320	10	10	5	b	b	b	b	b	0	1.54	0.09	5	
1000	10	10	5	b	b	b	b	b	0	1.45	0.19	5	
1000	10	10	5	b	b	b	b	b	0	1.39	0.18	5	1.42
1000	10	10	5	b	b	b	b	b	0	1.43	0.18	5	(0.02)
1000	10	10	5	b	b	b	b	b	0	1.41	0.12	5	

Emergence of seedlings was observed in the control on the third day.

The glass plate was removed on day 4.

Table B1.2a Data on emergence, condition, survival and wet-weight of Avena sativa exposed to TDA.

day	S 0	E 4	E 5	R 5	E 7	R 7	C[1)] 7	C[1)] 8	C[1)] 12	C[1)] 15	C[1)] 17	M 17	weight (g) mean	s.d.	N	average weight (s.d.) [3)]
conc. mg.kg^{-1}																
0	10	10	10	5	10	0	a	a	a	a	a	0	1.06	0.07	5	
0	10	10	10	5	10	0	a	a	a	a	a	0	0.94	0.17	5	0.97
0	10	10	10	5	10	0	a	a	a	a	a	0	0.88	0.19	5	(0.08)
0	10	10	10	5	10	0	a	a	a	a	a	0	0.98	0.08	5	
10	10	10	10	5	10	0	b	b	b	b	b	0	0.94	0.11	5	
10	10	10	10	5	10	0	b	b	b	b	b	0	0.84	0.12	5	0.92
10	10	10	10	5	10	0	b	b	b	b	b	0	0.96	0.09	5	(0.05)
10	10	10	10	5	10	0	b	b	b	b	b	0	0.93	0.11	5	
32	10	10	10	5	10	0	b	b	b	b	b	0	0.96	0.22	5	
32	10	10	10	5	10	0	b	b	b	b	b	0	0.96	0.13	5	0.95
32	10	10	10	5	10	0	b	b	b	b	b	0	0.92	0.09	5	(0.03)
32	10	10	10	5	10	0	b	b	b	b	b	0	0.98	0.02	5	
100	10	10	10	5	10	0	b	b	b	b	b	0	0.94	0.10	5	
100	10	10	10	5	10	0	b	b	b	b	b	0	0.96	0.10	5	0.97
100	10	10	10	5	10	0	b	b	b	b	b	0	1.02	0.10	5	(0.03)
100	10	10	10	5	10	0	b	b	b	b	b	0	0.95	0.11	5	
320	10	9	9	4	9	0	b	b	b	b	b	0	0.95	0.06	5	
320	10	10	10	5	10	0	b	b	b	b	b	0	1.01	0.08	5	0.97
320	10	7	7	2	7	0	b	b	b	b	b	0	0.97	0.13	5	(0.03)
320	10	10	10	5	10	0	b	b	b	b	b	0	0.97	0.15	5	
1000	10	0	6	1	6	0	d	d	d	d	d	0	0.22	0.13	5	
1000	10	0	2	0	2	0	d	e	e	f	d	0	0.11	0.08	2	0.16**
1000	10	0	3	0	6	1	d	d	d	d	d	0	0.15	0.06	5	(0.05)
1000	10	0	3	0	4	0	d	d	d	d	n	0	0.15	0.10	3	

Emergence of seedlings was observed in the control on the third day.

The glass plate was removed on day 3.

Table B1.2b Data on emergence, condition, survival and wet-weight of Lactuca sativa exposed to TDA.

day	S 0	E 4	E 5	R 5	E 7	R 7	C[1] 7	C[1] 8	C[1] 12	M 12	C[1] 15	C[1] 17	M 17	weight (g) mean	s.d.	N	average weight (s.d.) [3]
conc. mg.kg^{-1}																	
0	10	10	10	5	10	0	a	a	a	0	a	a	0	1.50	0.16	5	
0	10	10	10	5	10	0	a	a	a	0	a	a	0	1.26	0.16	5	1.36
0	10	10	10	5	10	0	a	a	a	0	a	a	0	1.38	0.12	5	(0.11)
0	10	10	10	5	10	0	a	a	a	0	a	a	0	1.29	0.35	5	
10	10	10	10	5	10	0	b	b	b	0	b	b	0	1.30	0.09	5	
10	10	10	10	5	10	0	b	b	b	0	b	b	0	1.33	0.39	5	1.35
10	10	10	10	5	10	0	b	b	b	0	b	b	0	1.51	0.19	5	(0.11)
10	10	10	10	5	10	0	b	b	b	0	b	b	0	1.27	0.15	5	
32	10	10	10	5	10	0	b	b	b	0	b	b	0	1.33	0.21	5	
32	10	10	10	5	10	0	b	b	b	0	b	b	0	1.46	0.19	5	1.41
32	10	10	10	5	10	0	b	b	b	0	b	b	0	1.46	0.26	5	(0.07)
32	10	10	10	5	10	0	b	b	b	0	b	b	0	1.36	0.23	5	
100	10	10	10	5	10	0	b	b	b	0	b	b	0	1.55	0.13	5	
100	10	10	10	5	10	0	b	b	b	0	b	b	0	1.35	0.22	5	1.38
100	10	10	10	5	10	0	b	b	b	0	b	b	0	1.29	0.21	5	(0.12)
100	10	10	10	5	10	0	b	b	b	0	b	b	0	1.34	0.10	5	
320	10	6	7	2	7	0	c	c	c	0	c	c	0	0.95	0.28	5	
320	10	7	8	3	8	0	c	c	c	0	c	c	0	0.86	0.13	5	0.93**
320	10	10	10	5	11[2]	1	c	c	c	0	c	c	0	0.91	0.06	5	(0.06)
320	10	5	7	2	7	0	c	c	c	0	c	c	0	0.98	0.07	5	
1000	10	0	9	4	9	0	k	k	l	5	l	l	5	--	--	0	
1000	10	0	6	1	6	0	k	k	l	5	l	l	5	--	--	0	0.01**
1000	10	0	7	2	7	0	k	k	m	4	m	m	4	0.01	--	1	(--)
1000	10	0	4	0	4	0	k	k	l	4	l	l	4	--	--	0	

Emergence of seedlings was observed in the control on the third day.

The glass plate was removed on day 3.

Table B1.3a Data on emergence, condition, survival and wet-weight of Avena sativa exposed to MDI.

day	S 0	E 3	E 4	R 4	C[1] 9	C[1] 10	C[1] 15	C[1] 16	C[1] 17	M 17	weight (g) mean	s.d.	N	average weight (s.d.) [3]
0	10	10	10	5	a	a	a	a	a	0	0.93	0.10	5	
0	10	10	10	5	a	a	a	a	a	0	0.91	0.20	5	0.93
0	10	8	10	5	a	a	a	a	a	0	0.96	0.09	5	(0.02)
0	10	10	10	5	a	a	a	a	a	0	0.93	0.13	5	
1000	10	10	10	5	b	b	b	b	b	0	0.87	0.09	5	
1000	10	10	10	5	b	b	b	b	b	0	0.88	0.10	5	0.89
1000	10	7	9	4	b	b	b	b	b	0	0.89	0.08	5	(0.03)
1000	10	9	10	5	b	b	b	b	b	0	0.93	0.11	5	

Emergence of seedlings was observed in the control on the third day.
The glass plate was removed on day 3.

Table B1.3b Data on emergence, condition, survival and wet-weight of Lactuca sativa exposed to MDI.

day	S 0	E 3	R 4	C[1] 9	C[1] 10	C[1] 15	C[1] 16	C[1] 17	M 17	weight (g) mean	s.d.	N	average weight (s.d.) [3]
0	10	10	5	a	a	a	a	a	0	1.19	0.12	5	
0	10	10	5	a	a	a	a	a	0	1.15	0.13	5	1.16
0	10	10	5	a	a	a	a	a	0	1.13	0.11	5	(0.03)
0	10	10	5	a	a	a	a	a	0	1.18	0.25	5	
1000	10	10	5	b	b	b	b	b	0	1.31	0.16	5	
1000	10	10	5	b	b	b	b	b	0	1.28	0.14	5	1.26
1000	10	10	5	b	b	b	b	b	0	1.16	0.18	5	(0.07)
1000	10	10	5	b	b	b	b	b	0	1.12	0.33	5	

Emergence of seedlings was observed in the control on the third day.
The glass plate was removed on day 4.

Table B1.4a Data on emergence, condition, survival and wet-weight of Avena sativa exposed to MDA.

day	S 0	E 5	R 5	C 5	E 7	R 7	C[1)] 7	C[1)] 8	C[1)] 14	C[1)] 15	C[1)] 17	M 17	weight (g) mean	weight (g) s.d.	weight (g) N	average weight (s.d.) [3)]
conc. mg.kg^{-1}																
0	10	8	3	a	10	2	a	a	a	a	a	0	0.95	0.14	5	
0	10	10	5	a	10	0	a	a	a	a	a	0	0.89	0.09	5	0.93
0	10	9	4	a	9	0	a	a	a	a	a	0	0.96	0.19	5	(0.03)
0	10	10	5	a	10	0	a	a	a	a	a	0	0.93	0.05	5	
10	10	10	5	b	10	0	b	b	b	b	b	0	0.97	0.10	5	
10	10	10	5	b	10	0	b	b	b	b	b	0	0.99	0.08	5	0.96
10	10	10	5	b	10	0	b	b	b	b	b	0	1.00	0.12	5	(0.05)
10	10	10	5	b	10	0	b	b	b	b	b	0	0.89	0.18	5	
32	10	10	5	b	10	0	b	b	b	b	b	0	0.88	0.17	5	
32	10	10	5	b	10	0	b	b	b	b	b	0	0.99	0.15	5	0.94
32	10	10	5	b	10	0	b	b	b	b	b	0	0.93	0.18	5	(0.05)
32	10	10	5	b	10	0	b	b	b	b	b	0	0.96	0.09	5	
100	10	10	5	b	10	0	b	b	b	b	b	0	1.00	0.08	5	
100	10	10	5	b	10	0	b	b	b	b	b	^	0.96	0.12	5	0.97
100	10	10	5	b	10	0	b	b	b	b	b	0	0.97	0.05	5	(0.02)
100	10	10	5	b	10	0	b	b	b	b	b	0	0.95	0.04	5	
320	10	10	5	c	10	0	c	c	c	c	c	0	0.53	0.09	5	
320	10	9	4	c	9	0	c	c	c	c	c	0	0.51	0.04	5	0.54**
320	10	9	4	c	9	0	c	c	c	c	c	0	0.54	0.08	5	(0.02)
320	10	10	5	c	10	0	c	c	c	c	c	0	0.56	0.03	5	
1000	10	9	4	i	9	0	h	h	h	h	h	0	0.07	0.02	5	
1000	10	7	2	i	7	0	h	h	h	h	h	0	0.08	0.02	5	0.07**
1000	10	6	1	i	9	3	h	h	h	h	h	0	0.06	0.02	5	(0.02)
1000	10	5	0	i	7	2	h	h	h	h	h	0	0.05	0.02	5	

Emergence of seedlings was observed in the control on the third day.

The glass plate was removed on day 3.

Table B1.4b Data on emergence, condition, survival and wet-weight of Lactuca sativa exposed to MDA.

day	S 0	E 5	R 5	C[1] 7	E 8	C[1] 8	E 14	R 14	C[1] 14	C[1] 15	C[1] 17	M 17	weight (g) mean	s.d.	N	average weight (s.d.) [3]
conc. mg.kg^{-1}																
0	10	10	5	a	10	a	10	5	a	a	a	0	1.37	0.12	5	
0	10	10	5	a	10	a	10	5	a	a	a	0	1.38	0.25	5	1.37
0	10	10	5	a	10	a	10	5	a	a	a	0	1.39	0.23	5	(0.02)
0	10	10	5	a	10	a	10	5	a	a	a	0	1.34	0.21	5	
3.2	10	10	5	b	10	b	10	5	b	b	b	0	1.43	0.13	5	
3.2	10	10	5	b	10	b	10	5	b	b	b	0	1.43	0.20	5	1.37
3.2	10	11[2]	6	b	11	b	11	6	b	b	b	0	1.30	0.09	5	(0.07)
3.2	10	10	5	b	10	b	10	5	b	b	b	0	1.32	0.16	5	
10	10	10	5	b	10	b	10	5	b	b	b	0	1.41	0.09	5	
10	10	10	5	b	10	b	10	5	b	b	b	0	1.36	0.14	5	1.42
10	10	10	5	b	10	b	10	5	b	b	b	0	1.46	0.10	5	(0.04)
10	10	10	5	b	10	b	10	5	b	b	b	0	1.44	0.12	5	
32	10	10	5	g	10	g	10	5	g	g	g	0	1.05	0.10	5	
32	10	10	5	b	10	b	10	5	b	b	b	0	1.25	0.31	5	1.23*
32	10	10	5	b	10	b	10	5	b	b	b	0	1.46	0.11	5	(0.17)
32	10	10	5	g	10	g	10	5	g	g	g	0	1.17	0.19	5	
100	10	10	5	h	10	h	10	5	c	c	c	0	1.05	0.06	5	
100	10	10	5	h	10	h	10	5	c	c	c	0	1.02	0.12	5	0.90**
100	10	10	5	h	10	h	10	5	c	c	c	0	0.69	0.12	5	(0.17)
100	10	10	5	h	10	h	10	5	c	c	c	0	0.93	0.10	5	
320	10	0	0	i	2	j	7	2	h	h	h	0	0.07	0.04	5	
320	10	1	0	i	1	j	6	1	h	h	h	0	0.13	0.09	5	0.12**
320	10	2	0	i	2	j	9	4	h	h	h	0	0.16	0.09	5	(0.04)
320	10	2	0	i	3	j	8	3	h	h	h	0	0.12	0.06	5	

Emergence of seedlings was observed in the control on the third day.

The glass plate was removed on day 5.

1) Explanation of the codes used in the description of the test plants
   - a: appearance of the plants normal (control, visually estimated)
   - b: appearance of the plants equal to that of the control plants
   - c: appearance of the plants not equal to that of the control, they are smaller.
   - d: appearance of the plants not equal to that of the control, they are very small and dark-green.
   - e: appearance of the plants not equal to that of the control. Of the two remaining plants, one seemed death at this moment in the test, the other was very small and dark-green
   - f: appearance of the plants not equal to that of the control, they are much smaller. The plant which was classified as dead on day 8 and 12 appeared to be still alive.
   - g: appearance of the plants not equal to that of the control, they are less regular in size than in the control.
   - h: appearance of the plants not equal to that of the control, they are much smaller.
   - i: appearance of the plants not equal to that of the control, they are much smaller and only very few seedlings had emerged.
   - j: appearance of the plants not equal to that of the control, they are much smaller, some of the seedlings seemed to be on the verge of emergence.
   - k: appearance of the plants not equal to that of the control, they are very small and most of them seemed to be dead.
   - l: the plants are dead.
   - m: appearance of the plants not equal to that of the control, four plants are dead, the fifth is very small
   - n: appearance of the plants not equal to that of the control, they are very small and dark green. One of the plants was not weighed after harvesting; it was probably dead.

2) Emergence of 11 plants was observed. Probably, 11 seeds were sown accidentally in these test cups.

3) Standard deviation of the mean weight per test cup.

* wet-weight is significantly different from that of the control plants (two-tailed Dunnett test, all plants are assumed to be independent, p=0.95).

** wet-weight is significantly different from that of the control plants (two-tailed Dunnett test, all plants are assumed to be independent, p=0.99).

Table B2 Individual wet-weights of the plants (in g) at the end of the experiment (t-17 days).

Table B2.1a Data on the wet-weight of Avena sativa exposed to TDI.

concentration ($mg.kg^{-1}$ soil)	cup	wet-weight (g) per plant at the end of the experiment (t=17).				
0	A	0.884	0.920	0.939	1.417	0.908
	B	1.061	1.047	0.954	1.005	0.800
	C	0.775	1.011	0.953	0.890	1.011
	D	0.935	0.946	0.928	1.067	0.918
1000	A	0.825	0.805	1.112	1.114	0.995
	B	0.973	1.257	0.632	0.882	1.084
	C	0.785	1.025	1.024	1.084	1.044
	D	1.057	0.762	1.128	1.055	1.075

Table B2.1b Data on the wet-weight of Lactuca sativa exposed to TDI.

concentration ($mg.kg^{-1}$ soil)	cup	wet-weight (g) per plant at the end of the experiment (t=17).				
0	A	1.598	1.472	1.311	1.194	1.429
	B	1.244	1.022	1.375	1.791	1.202
	C	1.672	1.234	1.562	1.288	1.189
	D	1.487	1.506	1.347	1.229	1.925
320	A	1.147	1.238	1.649	1.474	1.456
	B	1.545	1.212	1.240	1.337	1.459
	C	1.495	1.698	1.146	1.189	1.598
	D	1.493	1.660	1.603	1.491	1.454
1000	A	1.702	1.294	1.394	1.591	1.262
	B	1.447	1.097	1.475	1.374	1.571
	C	1.417	1.610	1.189	1.591	1.341
	D	1.503	1.552	1.400	1.244	1.364

Table B2.2a Data on the wet-weight of Avena sativa exposed to TDA.

concentration ($mg.kg^{-1}$ soil)	cup	wet-weight (g) per plant at the end of the experiment (t=17).				
0	A	1.130	1.049	0.972	1.040	1.133
	B	1.046	0.849	0.918	1.177	0.727
	C	0.626	0.953	1.140	0.800	0.890
	D	1.000	0.864	0.984	1.077	0.998
10	A	0.962	1.046	0.794	1.024	0.864
	B	0.846	0.726	1.031	0.755	0.852
	C	0.936	0.973	0.938	1.094	0.860
	D	0.772	0.873	0.933	1.066	0.982
32	A	0.600	1.092	0.977	1.176	0.936
	B	1.080	0.947	0.839	1.100	0.822
	C	0.844	0.828	0.914	0.960	1.062
	D	0.995	0.981	0.987	1.003	0.949
100	A	0.897	0.998	0.800	0.956	1.056
	B	0.935	0.788	1.015	1.057	0.998
	C	1.031	0.993	0.990	0.892	1.175
	D	0.866	0.882	0.865	1.021	1.114
320	A	0.987	1.022	0.932	0.903	0.891
	B	1.101	1.019	1.084	0.931	0.918
	C	0.995	1.049	0.965	1.082	0.751
	D	1.010	1.151	0.857	1.038	0.777
1000	A	0.021	0.239	0.222	0.205	0.400
	B	0.161	0.053			
	C	0.215	0.149	0.137	0.199	0.073
	D	0.178	0.232	0.042		

Table B2.2b Data on the wet-weight of Lactuca sativa exposed to TDA.

concentration ($mg.kg^{-1}$ soil)	cup	wet-weight (g) per plant at the end of the experiment (t=17).				
0	A	1.438	1.617	1.250	1.587	1.612
	B	1.415	1.447	1.242	1.074	1.140
	C	1.259	1.314	1.586	1.374	1.347
	D	1.044	1.248	1.834	1.384	0.935
10	A	1.212	1.456	1.305	1.238	1.306
	B	1.272	2.014	1.191	1.123	1.041
	C	1.768	1.333	1.543	1.596	1.300
	D	1.048	1.390	1.244	1.421	1.223
32	A	1.365	1.050	1.236	1.612	1.387
	B	1.543	1.368	1.604	1.172	1.629
	C	1.048	1.540	1.455	1.768	1.509
	D	1.300	1.583	1.267	1.057	1.608
100	A	1.334	1.636	1.534	1.670	1.596
	B	1.355	1.563	1.300	1.518	0.995
	C	1.152	1.528	1.152	1.092	1.506
	D	1.311	1.387	1.216	1.481	1.300
320	A	0.952	0.755	1.243	1.215	0.609
	B	0.973	0.656	0.914	0.783	0.955
	C	0.920	0.814	0.887	0.953	0.960
	D	1.006	1.063	0.991	0.977	0.880
1000	A					
	B					
	C	0.012				
	D					

Table B2.3a Data on the wet-weight of Avena sativa exposed to MDI.

concentration ($mg.kg^{-1}$ soil)	cup	wet-weight (g) per plant at the end of the experiment (t=17).				
0	A	0.769	0.953	0.984	1.046	0.909
	B	1.105	0.923	0.650	1.091	0.782
	C	1.040	1.045	0.847	0.973	0.907
	D	0.785	1.108	0.847	1.024	0.873
1000	A	0.766	0.806	0.979	0.859	0.934
	B	0.920	0.980	0.782	0.949	0.769
	C	0.772	0.896	0.980	0.936	0.849
	D	0.976	1.049	0.741	0.927	0.946

Table B2.3b Data on the wet-weight of Lactuca sativa exposed to MDI.

concentration ($mg.kg^{-1}$ soil)	cup	wet-weight (g) per plant at the end of the experiment (t=17).				
0	A	1.098	1.062	1.199	1.250	1.364
	B	1.212	0.984	1.332	1.070	1.136
	C	1.141	1.104	1.212	1.245	0.951
	D	1.576	1.282	1.038	1.017	0.990
1000	A	1.398	1.341	1.015	1.404	1.375
	B	1.174	1.501	1.162	1.307	1.247
	C	1.098	1.332	0.940	1.363	1.045
	D	0.859	1.256	1.735	1.250	1.492

Table B2.4a Data on the wet-weight of Avena sativa exposed to MDA.

concentration ($mg.kg^{-1}$ soil)	cup	wet-weight (g) per plant at the end of the experiment (t=17).				
0	A	1.067	0.816	1.065	0.791	1.017
	B	0.831	1.012	0.916	0.920	0.765
	C	0.746	0.918	1.099	0.828	1.190
	D	0.920	0.989	0.942	0.850	0.964
10	A	0.812	1.055	0.973	1.057	0.974
	B	1.018	1.014	1.006	0.850	1.071
	C	1.106	1.105	0.953	0.809	1.009
	D	0.721	0.745	1.042	0.823	1.134
32	A	0.515	0.896	0.887	0.911	1.094
	B	0.849	1.167	1.134	0.894	0.898
	C	0.631	0.941	0.941	1.070	1.057
	D	0.837	1.055	0.997	1.016	0.909
100	A	1.018	0.970	1.037	0.885	1.102
	B	0.909	0.884	0.827	1.079	1.106
	C	0.939	0.933	0.940	1.043	1.013
	D	0.889	0.996	0.944	0.963	0.937
320	A	0.553	0.550	0.625	0.385	0.561
	B	0.489	0.493	0.582	0.484	0.520
	C	0.626	0.531	0.412	0.540	0.576
	D	0.520	0.587	0.536	0.593	0.587
1000	A	0.071	0.044	0.088	0.062	0.075
	B	0.106	0.088	0.093	0.081	0.057
	C	0.078	0.056	0.036	0.085	0.062
	D	0.024	0.044	0.070	0.035	0.068

Table B2.4b Data on the wet-weight of Lactuca sativa exposed to MDA.

concentration ($mg.kg^{-1}$ soil)	cup	wet-weight (g) per plant at the end of the experiment (t=17).				
0	A	1.293	1.445	1.358	1.523	1.213
	B	1.404	1.422	1.702	1.343	1.006
	C	1.641	1.377	1.558	1.049	1.304
	D	1.375	0.968	1.455	1.509	1.395
3.2	A	1.377	1.293	1.340	1.609	1.515
	B	1.218	1.728	1.435	1.472	1.299
	C	1.184	1.336	1.294	1.240	1.430
	D	1.215	1.477	1.164	1.504	1.258
10	A	1.302	1.493	1.483	1.343	1.444
	B	1.459	1.153	1.298	1.371	1.524
	C	1.513	1.376	1.371	1.613	1.424
	D	1.372	1.591	1.281	1.480	1.464
32	A	1.070	1.202	1.024	0.939	1.037
	B	1.276	1.467	1.417	0.713	1.359
	C	1.597	1.414	1.331	1.532	1.414
	D	1.173	1.062	0.913	1.431	1.247
100	A	1.062	1.110	1.011	1.096	0.966
	B	1.122	0.855	1.114	0.922	1.077
	C	0.566	0.622	0.812	0.824	0.617
	D	0.755	0.704	0.852	0.885	0.940
320	A	0.100	0.128	0.044	0.035	0.043
	B	0.082	0.290	0.140	0.045	0.102
	C	0.175	0.186	0.302	0.046	0.115
	D	0.075	0.198	0.172	0.068	0.095

ANNEX C COMPOSITION OF THE SOIL/SAND MIXTURE

Composition of mineral particles				%
0	-	2	µm	6.6
2	-	16	µm	4.1
16	-	50	µm	7.4
50	-	105	µm	8.0
105	-	150	µm	16.7
150	-	2000	µm	57.2

In % of dry soil

organic matter	1.1
$CaCO_3$	5.1
Silt 0 - 16 µm	10.1
Sand 16 - 2000 µm	83.7
pH-KCl	7.6

Data determined at 'Bedrijfslaboratorium voor grond- en gewasonderzoek' (not under GLP conditions).

CERTIFICATE OF AUTHENTICITY

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